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*The University of Minnesota*
*Agricultural Experiment Station*

# *Studies on the Parasitism of Helminthosporium Sativum*

*By J. J. Christensen*
*Division of Plant Pathology and Botany*



UNIVERSITY FARM, ST. PAUL

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The figures in Tables XII, XIV, and XV are centimeters instead of cubic centimeters, as given.

The description of Fig 2, Pl. III should read:

A. *Hordeum murinum*
Check
Inoculated

B. *Agropyron tenerum*
Check
Inoculated

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# STUDIES ON THE PARASITISM OF HELMINTHOSPORIUM SATIVUM

By J. J. Christensen[1]

## INTRODUCTION

Within the last three or four years increasing importance has been attached to cereal diseases caused by *Helminthosporium sativum* Pammel, King, and Bakke.

In 1909 Pammel (5) published a short article on a new disease of barley characterized by irregular brownish lesions on the leaves. This was followed the next year by a publication by Pammel, King, and Bakke (6) in which they attributed the cause of the spot disease to *Helminthosporium sativum n. sp.*

Bolley (1), in 1913, called attention to the seriousness of root- and foot-rots of wheat and stated that constant cropping of wheat on the same land caused wheat sickness and wheat-sick soil. He maintained that this sickness of soil was not due to lack of essential elements or to permanent excrement or poisons detrimental to plants. He stated that it was caused by fungi, especially Helminthosporium, Alternaria, Fusarium, and Colletotrichum.

In the spring of 1919 pathologists and numerous wheat growers became greatly concerned over a serious and destructive foot- and root-rot of wheat which occurred in Illinois and Indiana. This was at first thought to be the Australian Take-All, which is caused by *Ophiobolus cariceti* (B. and Br.) Sacc. However, Stevens (10) was of the opinion that the disease might be caused by *Helminthosporium sp.*

Louise J. Stakman (9), in 1920, showed that *Helminthosporium sp.*, apparently identical with *H. sativum*, caused not only spot blotch of barley, but also root-rot and seedling blight of wheat and rye. By artificial inoculations, she proved that the same fungus also readily attacked various grasses.

Hamblin (3) was of the opinion that the "foot-rot" caused by *Helminthosporium sp.* did more damage to the wheat in Australia in 1921 than did the true Take-All.

The diseases caused by *Helminthosporium sativum* appear to be very widely distributed, as inoculations made from cultures obtained from different sections of the United States, Canada, Argentina, and

[1] The writer is greatly indebted to Dr. E. C. Stakman, under whose direction the work was done, for much helpful criticism throughout the work; and to Mr. Arthur Henry and Dr. Louise Dosdall of the University of Minnesota; Mr. C. O. Hamblin, Assistant Biologist of New South Wales; and Dr. F. L. Stevens, of the University of Illinois, for cultures of the organism.

Australia produce typical symptoms on wheat, emmer, barley, and rye. The same organism was also isolated from wheat grown in Mexico. During the summer of 1921 the pathogene was isolated from specimens from North Dakota, Nebraska, Oklahoma, Texas, New Mexico, Iowa, Wisconsin, Michigan, Illinois, West Virginia, and Minnesota.

In 1919 the disease was reported from practically every important wheat growing county in Minnesota. In some localities it caused considerable losses. Some fields of wheat in Sherburne county were so badly injured that the farmers plowed them under and planted them to corn. In 1921 the disease again was serious and widespread in the state. The writer visited fields of wheat, rye, and barley in twenty important grain-growing counties. Infection was common in every field inspected. Severe infection and considerable damage were observed in several counties, especially in St. Louis, Anoka, Kittson, Pennington, Marshall, and Wilkin; and specimens were received from several other counties. In the spring of 1922 it was estimated that the disease caused a loss of from ten to twenty per cent in many fields of barley and wheat in Dakota, McLeod, and Rice counties. Heavily infected fields also were observed in Renville, Meeker, Chippewa, Polk, and Scott counties. The damage was manifested by local necrosis of the leaves and by a stunted, spindling, or rosette appearance of the infected plants and a marked rotting of the root system.

The pathogene, *Helminthosporium sativum*, then, is responsible for leaf spot, root-rot, foot-rot, and seedling blight of wheat, barley, rye, and numerous grasses. The disease caused by it apparently is becoming more widely distributed and more destructive, not only in Minnesota and other parts of the United States but also in many of the other important grain-growing countries of the world. For these reasons, it is desirable to know more about the parasitism of the fungus. The investigations reported in this bulletin include a study of the host range and biologic specialization of the fungus, the varietal resistance of its hosts, methods of overwintering, and sources of infection.

## SYMPTOMS

*Helminthosporium sativum* attacks every part of a susceptible host. Roots, stems, leaves, spike, spikelets, and seeds all become infected. The symptoms described here have not only been observed in the field but have been produced by artificial inoculations in the greenhouse.

The first apparent symptom of the disease in the fields is a seedling blight somewhat like damping-off. The plants may be so severely attacked by the fungus at the ground line that they are quickly killed. Some of the diseased seedlings may even fail to push out of the soil, their roots and young shoots being completely rotted by the organism.

Others may develop a fairly good root system but the stems are destroyed. Still other plants may develop one or two healthy leaves, but soon die because they fail to produce roots (see Plate II). If the seeds are severely infected they may not germinate; while, if the infection is less severe, weak and spindling plants may develop.

However, the most conspicuous symptom of the disease is the distinct dwarfing of the infected plants. In heavily infected areas these stunted plants often occur in characteristic patches which are usually circular but frequently irregular in outline, and which may vary from a few feet to several rods in diameter. Similar dwarfed plants also may be found intermixed with healthy plants.

Often the basal leaves of these stunted plants arise at the ground line or even below it and they often are darker green in color than normal ones. The internodes and the first leaves are considerably shorter than those of normal plants, the primary roots are infected, and the foot of the plant is discolored by the fungus. Large chocolate-colored lesions often occur at the base of the first leaf. Occasionally one or more large spots appear on the blades of the first and second leaves, and curling of these leaves is not uncommon. Infected plants may recover and grow to maturity, or they may gradually succumb to the pathogene.

If the seedlings are not killed, the leaves of the diseased plant may become a darker green than those of normal plants. Severely infected plants usually remain dwarfed and may stool excessively. As many as thirty to forty culms are not uncommon, especially on diseased barley plants; but, as a rule, not more than half that many develop. In many cases only one or two of the culms develop normally and produce seeds (see Plate III, Fig. 1). Sometimes, however, the same organism prevents stooling by attacking the new shoots before they emerge from the sheath, or soon after. In fact, this is one of the most common symptoms observed in the wheat fields of Minnesota.

Likewise, the secondary roots are frequently infected while quite small. This results in a poor root system, in foot-rot, and in weak, spindling plants. The roots are brittle and decayed, and on attempting to pull them up, they often break at the crown and remain in the soil. Diseased plants reach varying stages of development and maturity, depending on the degree of infection and on environmental conditions. Owing to reduced root systems the nutrition of the plant is interfered with and the water supply is cut down. The heads may be poorly filled and the seeds are sometimes shriveled.

The symptoms thus far described may all result from seed or soil infection. Subsequently secondary infections also may occur. On the

leaves, these infections usually appear as numerous dark-brown, oval or irregular blotches (see Plate I). The appearance of the lesions, however, varies with the host. For instance, on einkorn and on *Bromus villosus* Forsh, the lesions have a light center bordered by a halo; and on certain varieties of rye a water-soaked leaf spot, with or without a brownish border, is produced. On *Andropogon sorghum* Brot. red, oval lesions develop, while on *Agropyron repens* (L.) Beauv. the lesions are usually elongated and black. Sometimes two or even more types of infection appear on the same leaf. The size of the lesions varies greatly on the same leaf, ranging from very small to 3 by 20 millimeters or more in size. The coalescence of these spots often results in the death of part or all of the infected leaf.

The fungus also causes similar lesions on the stem, glumes, awns, and even on the seeds. The pathogene fruits very abundantly on the nodes, giving them a black velvety appearance. The internodes seldom become very much darkened, except near the base of the culms, altho occasionally all of them may be much discolored.

The seeds also may be discolored, or the entire head or portions of it may be blighted. The ovary of the floret may be attacked at any stage in its development, and the kernel either may fail to form, or, when formed, it may be badly shrunken. Generally the germ end of the seed is most conspicuously discolored. Under favorable conditions, the fungus fruits luxuriantly on undeveloped kernels. Sometimes the rachis is infected and this prevents proper filling of the parts above the lesion. The causal organism fruits very abundantly in nature on the glumes of the spikelets of wheat, emmer, spelt, and einkorn.

All the enumerated symptoms can be caused by the same fungus, *Helminthosporium sativum* Pammel, King, and Bakke. This organism was found to be constantly associated with the various lesions on the different parts of the plants. Hundreds of isolations were made from the infected plants collected, not only in Minnesota and adjoining states, but also in various parts of the United States and other countries. The fungus was isolated from many naturally infected cereals and grasses, including several varieties of rye and corn, 20 varieties of barley, and 70 varieties of wheat. In addition, the same, or at least a very similar organism, was isolated from the following grasses:

*Alopecurus pratensis* L., *Agropyron caninum* (L.) Beauv., *A. disertorum* Schult., *A. repens* (L.) Beauv., *A. smithii* Rydb., *A. tenerum* Vasey, *Andropogon furcatus* Muhl., *A. sorghum sudanensis* Piper, *Bromus inermis* Leyss., *Calamagrostis canadensis* (Michx.) Beauv., *Dactylis glomerata* L., *Digitaria sanguinalis* Scop., *Echinochloa crus-galli* (L.) Beauv., *Elymus canadensis* L., *E. glaucus* Buckl., *E. robustus*

Scribn. and J. G. Sm., *E. straitus* Willd., *E. virginicus* L., *Hordeum jubatum* L., *H. pusillum* Nutt., *Hystrix patula* Moench., *Lolium sp.* L., *Muhlenbergia sp.*, Schreb., *Panicum capillare* L., *Phalaris arundinacea* L., *Phragmites phragmites* L. Karst., *Setaria glauca* L., *S. italica* Beauv., *S. viridis* L., *Sorghum halapense* (L.) Pers., *Stipa spartea* Trin., and *Zizania palustris* L. The same fungus was re-isolated from several hundred plants which became diseased as a result of artificial inoculation in the field and greenhouse.

Cross-inoculations with several strains of Helminthosporium obtained from wheat, barley, and rye all produced the typical "spot-blotch" on barley and the same organism also infected wheat, rye, and many grasses.

Plants were grown in more than 600 pots of soil which had been inoculated with *Helminthosporium sativum* and in addition, thousands of plants representing many genera, species, and varieties were sprayed with spore suspensions. By this means the symptoms which have been described were obtained repeatedly.

Thus, the disease was produced in both the field and the greenhouse by inoculating seed and soil, and by spraying the plants with a suspension of spores. In the laboratory, infected seeds developed diseased seedlings when germinated on agar (see Plate IV, Fig. 1) or on sterile blotting paper, and in the greenhouse by planting them in sterile sand or loam. The disease also was reproduced in the greenhouse by using soil from fields where severe infection had occurred.

## CULTURAL CHARACTERS AND BIOLOGICAL SPECIALIZATION

No general description of the morphological or cultural characteristics can be given because transfers from the same culture produce different types of growth and wide variation in size of spores on different media and under different environmental conditions. Moreover, there are several physiological races or biologic forms of *Helminthosporium sativum*, each of which produces a different type of growth on a given medium.

While studying cultural characteristics of two strains of *Helminthosporium sativum* derived from single spores isolated from spot blotch on barley, it was observed that marked variations occurred when they were grown on the same medium under the same conditions. For this reason the characteristics of two cultures from barley, one from rye, and one from wheat, all derived from single spores, were compared on green bean agar, on one per cent potato dextrose agar, and on carrot agar. These comparative studies were made in petri dishes

of the same diameter. Tubes from which the plates were poured contained ten cubic centimeters of medium, and two tubes were poured into each petri dish. Sixteen plates of each kind of medium were used, four of each being inoculated with each culture of *Helminthosporium sativum*. Inoculations were made from cultures of the same age, and only one tube of each culture was used in inoculating the twelve plates of the three different media. The inoculum consisted of a small portion of the potato agar containing mycelium and spores, and as nearly as possible the same amount was used in each case. The plates were placed in a dark incubator, in which the temperature varied between 20° and 22°C. The fungus was permitted to grow for three or four days before any notes were taken, in order to eliminate any effect due to differences in the amount of inoculum in the different cultures.

It is evident from Plates V and VI and from Table I that the cultural characters of the four biologic forms greatly differ from each other on the same medium. The cultural characters of the same form are also different on different media, for instance, Form IV produces a white growth on green bean agar, but a black colony on potato dextrose agar. It was repeatedly observed that the color and nature of growth on a given medium varied with the temperature, amount of moisture, and other factors. But the colonies of the same form on the four plates were consistent. Thus the type of growth under the same conditions depends first on the form and second on the medium used. Plates V and VI and Table I indicate that two strains may appear similar when grown on one type of medium, but may differ markedly when transferred to a different medium. For instance, Forms I and IV are very similar on potato dextrose but are strikingly different on green bean agar.

As is brought out in Table I, there also is considerable variation in the rates of growth of the four cultures on the same and on different media. On potato dextrose Form I increased 3.44 centimeters in twenty-four hours, while Form II increased only 0.94 cm. in the same time. On green bean agar, however, the latter strain grew more than 5 cm. during the same period. Thus, the rate of growth at a uniform temperature depends on the kind of medium as well as on the form used.

Comparative inoculations were made with these four biologic forms on wheat, barley, rye, and grasses. All caused typical spot blotch on barley, but differed in the severity of infection which they produced on all the hosts. The exact differences in the pathogenicity of the four forms, however, can not be given definitely.

TABLE I

COMPARATIVE CULTURAL CHARACTERISTICS OF FOUR BIOLOGIC FORMS OF *H. sativum*, WHEN GROWN ON THE SAME AND DIFFERENT MEDIA UNDER THE SAME CONDITIONS

| Biologic forms | Rate of growth per day* | | | Color of colonies | | | Character of growth† | | |
|---|---|---|---|---|---|---|---|---|---|
| | Potato dextrose agar | Green bean agar | Carrot agar | Potato dextrose agar | Green bean agar | Carrot agar | Potato dextrose agar | Green bean agar | Carrot agar |
| | cm. | cm. | cm. | | | | | | |
| I Isolated from rye | 3.44 | 7.62 | 2.27 | Grayish black | Olive gray | Dark blue-green | Regular; very finely stippled; zonation sharp | Regular; few large zones | Very irregular |
| II Isolated from barley | 4.31 | 6.40 | 2.64 | Grayish black | Grayish black | Dark blue-green | Regular; coarsely stippled; zonation sharp | Regular; tufts common; zonation not as sharp as in Form I | Slightly irregular; considerable guttation |
| III Isolated from barley | 0.94 | 5.12 | 2.25 | Gray | White, black and gray intermixed | Grayish to dull yellow | Very irregular; no zonation | Irregular; cones of varied color; zonation not sharp | Irregular; considerable aerial mycelium |
| IV Isolated from wheat (Culture obtained from Dr. F. L. Stevens) | 3.56 | 7.06 | 1.44 | Grayish black | White | Dark blue-green | Slightly irregular; zonation very sharp very little aerial mycelium | Regular; white aerial mycelium; no sporulation | Dense; very little aerial mycelium |

* Average increase in diameter per day over a period of four days.

† Six to ten days after inoculations.

The facts that these cultures differ physiologically, as is indicated by their different reactions to the same medium under uniform conditions, and that they react differently on various hosts, proves that biologic specialization exists in *Helminthosporium sativum* and that the four cultures obtained from single spore isolations and used in this investigation must be regarded as four distinct biologic forms of this species.

The variation of one form under different conditions, or of different forms under the same conditions, is very marked. A technical description of the organism, therefore, is of little value, unless the exact conditions under which it was grown are definitely known.

## MORPHOLOGY OF THE SPORES

In the laboratory, Form I was found to sporulate very abundantly on boiled rice, wheat, barley, and green beans, and on one per cent potato dextrose agar. In nature, the fungus apparently fruits most luxuriantly on nodes of susceptible cereals. The conidia are usually solitary but they also have been observed in chains on potato dextrose agar. In cultures on potato dextrose agar, conidia were often seen germinating and giving rise to smaller ones; these in turn produced still smaller ones, and so on.

The conidia have a thin epispore dark-olive or brown in color, and a hyaline endospore. The shape is variable as is shown in Plate IV, Figures 2 and 3. The spores are typically spindle-shaped, with rounded ends, but frequently the widest point is nearer one end than the other. The shape ranges from fusoid to clavate or cylindric. Slight variations in shape occur among the spores of different forms and even among those of the same form with varying conditions of environment and substratum. The spores are often curved, and occasionally are irregular.

The number of septa in the spores varies with the biologic form and with the medium on which the fungus is grown. Two hundred spores of each form were observed. Table II summarizes the number of septa in the spores of the four biologic forms which were grown on heads of Marquis wheat under the same conditions. Table III gives similar data for Form I on different media. Each class in Tables II and III differs by one septum.

Table II indicates that the four forms differ in septation. The mode of Form IV is at eight septa, while that of Form III is at six. There is but little difference in the number of septa in spores of different biologic forms which have been grown under the same conditions. There is greater variation within a single form when grown under different environmental conditions. This is brought out by Table III.

TABLE II

VARIABILITY IN NUMBER OF SEPTA OF CONIDIA OF FOUR DIFFERENT BIOLOGIC FORMS OF *H. sativum* PRODUCED ON HEADS OF WHEAT UNDER THE SAME CONDITIONS

| Biologic forms | Septation classes | | | | | | | | | | | | Range | Mode |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | 0 | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 | |
| I | 3 | 0 | 0 | 3 | 15 | 19 | 37 | 52 | 39 | 20 | 10 | 2 | .. | 0–11 | 7 |
| II | .. | .. | 1 | 2 | 8 | 9 | 31 | 56 | 51 | 29 | 9 | 3 | 1 | 2–12 | 7 |
| III | 4 | 5 | 6 | 17 | 30 | 40 | 53 | 28 | 10 | 4 | 3 | .. | .. | 0–10 | 6 |
| IV | .. | .. | .. | 1 | 3 | 9 | 25 | 58 | 64 | 29 | 8 | 3 | .. | 3–11 | 8 |

TABLE III

VARIABILITY IN NUMBER OF SEPTA OF CONDIA OF BIOLOGIC FORM I OF *H. sativum* PRODUCED ON DIFFERENT MEDIA

| Culture medium | Septation classes | | | | | | | | | | | | Range | Mode |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | 0 | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | | |
| Head of wheat..... | 3 | 0 | 0 | 3 | 15 | 19 | 37 | 52 | 39 | 20 | 10 | 2 | 0–11 | 7 |
| Potato dextrose* (A)..... | 3 | 8 | 10 | 15 | 29 | 48 | 46 | 25 | 11 | 4 | 1 | .. | 0–10 | 5 |
| Potato dextrose (B)..... | 26 | 47 | 48 | 24 | 24 | 13 | 12 | 5 | 1 | .. | .. | .. | 0– 8 | 2 |

* Potato dextrose (A) and (B) were made up according to the same formula but at different times.

Table IV summarizes the lengths of five hundred spores for each of the four biologic forms of *Helminthosporium sativum* from cultures grown under the same conditions on heads of wheat. Table V gives the lengths of five hundred spores of Form I on different media. In Tables IV and V the spore lengths are grouped into classes, the range of each class being ten microns, and from these were computed the biometrical constants with their probable errors for the different biologic forms on the same medium and for Form I on different media.

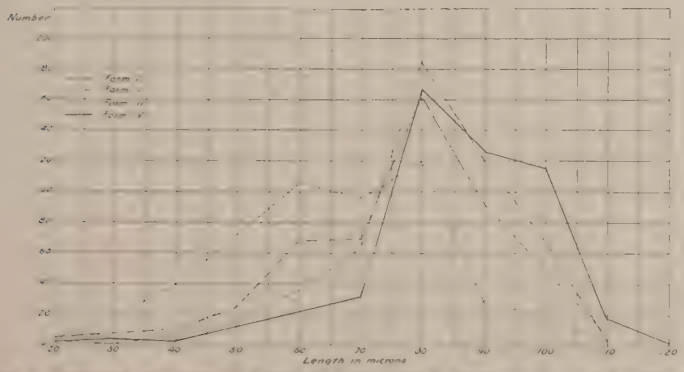


Fig. . Difference in Length of Spores of Four Biologic Forms of *H. sativum* Developed on Heads of Wheat Under the Same Condition

TABLE IV

VARIATIONS AND CONSTANTS FOR LENGTH OF SPORES OF BIOLOGIC FORMS OF *H. sativum* DEVELOPED ON HEADS OF WHEAT UNDER THE SAME CONDITIONS

| Biologic forms | Spore-length classes (in microns) | | | | | | | | | | | Range | Constants | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | 20 | 30 | 40 | 50 | 60 | 70 | 80 | 90 | 100 | 110 | 120 | | Mode | Means | Standard deviation | Coefficient variability |
| I | 5 | 8 | 10 | 25 | 68 | 69 | 163 | 93 | 50 | 9 | ... | 11.40—114.00 | 80 | 76.60± 0.51 | 16.86± 0.36 | 22.01± 0.47 |
| II | 1 | 0 | 6 | 17 | 36 | 65 | 184 | 119 | 69 | 3 | ... | 38.00—106.40 | 80 | 80.96± 0.52 | 13.50± 0.41 | 16.67± 0.36 |
| III | 3 | 19 | 41 | 74 | 105 | 96 | 120 | 30 | 10 | 2 | ... | 15.20—106.40 | 80 | 65.02± 0.50 | 16.65± 0.35 | 25.60± 0.55 |
| IV | 1 | 3 | 2 | 12 | 22 | 32 | 166 | 126 | 116 | 18 | 2 | 26.60—117.80 | 80 | 85.58± 0.43 | 14.16± 0.30 | 16.53± 0.35 |

TABLE V

VARIATIONS AND CONSTANTS FOR LENGTH OF SPORES OF BIOLOGIC FORM I OF *H. sativum* PRODUCED ON DIFFERENT MEDIA

| Culture medium | Spore-length classes (in microns) | | | | | | | | | | Length limits | Constants | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | 10 | 20 | 30 | 40 | 50 | 60 | 70 | 80 | 90 | 100 | 110 | | Mode | Means | Standard deviation | Coefficient variability |
| Head of wheat | ... | 5 | 8 | 10 | 25 | 68 | 69 | 163 | 93 | 50 | 9 | 15.20—114.00 | 80 | 76.60± 0.51 | 16.86± 0.36 | 22.01± 0.47 |
| Potato dextrose* (A) | ... | 24 | 41 | 21 | 96 | 161 | 85 | 62 | 8 | 1 | 1 | 15.20—114.00 | 60 | 57.70± 0.50 | 16.45± 0.35 | 28.50± 0.61 |
| Potato dextrose (B) | 3 | 169 | 197 | 40 | 42 | 18 | 13 | 14 | 3 | 1 | ... | 11.40—106.40 | 30 | 33.00± 0.47 | 15.62± 0.33 | 47.33± 1.09 |

* Potato dextrose (A) and (B) were made up according to the same formula, but at different times.

The spore lengths represented in Tables IV and V are plotted in Figures 1 and 2. All four forms have the same mode, namely, 80 microns. The modes in Table V and the curves in Figure 2 show at a glance that the length of spore of a single form varies greatly on different media. For example the modal length of Form I grown on a head of wheat is 80 microns, on potato dextrose in one case, 60 microns; in the other, 30 microns.

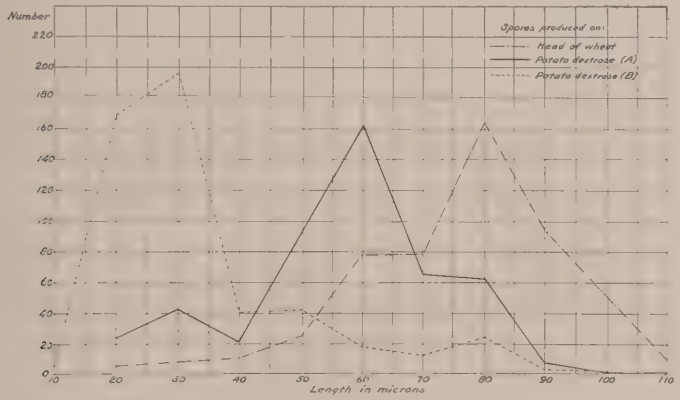


Fig. 2. Difference in Length of Spores of Form I of *H. sativum* Developed on Different Media

TABLE VI

SUMMARY OF DIFFERENCES IN THE MEAN LENGTH OF SPORES OF BIOLOGIC FORMS OF *H. sativum*

| Biologic forms | Difference in mean length (in microns) | Difference in mean length divided by probable error of the difference |
|---|---|---|
| I and II | 4.36±0.65 | 6.71 |
| I and III | 11.58±0.71 | 16.31 |
| I and IV | 8.98±0.67 | 13.40 |
| II and III | 15.94±0.65 | 24.52 |
| II and IV | 4.62±0.59 | 7.83 |
| III and IV | 20.56±0.66 | 31.51 |

TABLE VII

SUMMARY OF DIFFERENCES IN THE MEAN LENGTH OF THE SPORES OF BIOLOGIC FORM I OF *H. sativum* PRODUCED ON DIFFERENT MEDIA

| Culture medium | Difference in mean length (in microns) | Difference in mean length divided by probable error of the difference |
|---|---|---|
| Head of wheat and potato dextrose* (A)..... | 18.90±0.71 | 26.61 |
| Head of wheat and potato dextrose (B)..... | 43.60±0.69 | 63.18 |
| Potato dextrose (A) and Potato dextrose (B).. | 24.70±0.68 | 36.32 |

* Potato dextrose (A) and (B) were made up according to the same formula but at different times.

Table VI gives the differences in the mean lengths of conidia of the four biologic forms of *Helminthosporium sativum* and the ratios between these differences and their probable errors. Table VII summarizes similar data for Form I grown under different conditions. The differences between the mean lengths of the spores of the four biologic forms are significant in every case. The differences in mean spore length of the same Form I on different media, given in Table VII, are surely very significant. In one case the mean is more than sixty-three times as great as the probable error.

TABLE VIII

VARIABILITY IN WIDTH OF SPORES OF THE DIFFERENT BIOLOGIC FORMS OF *H. sativum* PRODUCED ON HEADS OF WHEAT UNDER THE SAME CONDITIONS

| Biologic forms | Spore-width classes (in microns) | | | | | | Range | Mode |
|---|---|---|---|---|---|---|---|---|
| | 11.40 | 15.20 | 19.00 | 22.80 | 26.60 | 30.40 | | |
| I | ..... | 4 | 60 | 190 | 44 | 2 | 15.20–30.40 | 22.80 |
| II | ..... | 1 | 27 | 194 | 77 | 1 | 15.20–30.40 | 22.80 |
| III | 2 | 39 | 150 | 94 | 15 | ..... | 11.40–26.60 | 19.00 |
| IV | 1 | 3 | 48 | 221 | 27 | ..... | 11.40–26.60 | 22.80 |

TABLE IX

VARIABILITY IN WIDTH OF SPORES OF BIOLOGIC FORM I OF *H. sativum* ON DIFFERENT MEDIA

| Culture medium | Spore-width classes (in microns) | | | | | | Range | Mode |
|---|---|---|---|---|---|---|---|---|
| | 11.40 | 15.20 | 19.00 | 22.80 | 26.60 | 30.40 | | |
| Head of wheat..... | ..... | 4 | 60 | 190 | 44 | 2 | 15.20–30.40 | 22.80 |
| Potato dextrose* (A)..... | 8 | 25 | 122 | 131 | 14 | ..... | 11.40–26.60 | 22.80 |
| Potato dextrose (B)..... | 78 | 121 | 63 | 34 | 4 | ..... | 11.40–26.60 | 15.20 |

* Potato dextrose (A) and (B) were made up according to the same formula, but at different times.

Table VIII summarizes the variation in width of spores of the different biologic forms. Three hundred spores were measured in every case, the spores being the same that were used for measurements of length. Table IX gives similar results for Form I when grown on heads of wheat and potato dextrose. The range of each class in Tables VIII and IX is 3.8 microns. It will be noticed that Form III, which had practically the same modal length as Form I, had a modal width 3.8 microns smaller than that of the other three forms. In Form I marked variation in width is evident between spores grown on a head of wheat and those grown on potato dextrose.

The size, shape, and degree of curvature of spores of the same form vary considerably on different media and under different conditions. This variation is so marked that one would be inclined to

consider the extreme cases not merely as different biologic forms of one species but rather as distinct morphological species. For example, the mode of Form I is 33 microns on potato dextrose (B), while on wheat heads it is 80 microns. A large number of spores must, therefore, be measured in order to obtain reliable results, and any given spore dimension can be applied only to spores produced on a specific medium, under known conditions. On potato dextrose (A) the measurements of Form I agree fairly well with those given by Louise J. Stakman (9). On the head of wheat they approach the measurements obtained by Pammel, King, and Bakke (6), but resemble more closely those mentioned by Stevens (10).

## PATHOGENICITY STUDIES

### METHODS AND MATERIALS

The organism used in the following experiments was isolated from rye seed unless otherwise indicated. Each strain of the fungus used was derived from a single spore isolation.

The fungus used for leaf inoculations was grown on potato dextrose agar. The spores were carefully scraped off into water and the suspension was poured through cheesecloth to remove any bits of medium that were accidentally loosened during the process. The spores were then applied with a small Daisy hand sprayer. The plants inoculated in the greenhouse were well moistened and special pains were taken to keep a constant film of water on them during incubation.

After inoculation, the plants were placed in a thoroly cleaned incubation chamber for about seventy hours. These chambers consisted of large galvanized iron cylinders with glass tops and fitted closely into a pan which contained an inch or two of water. During warm, sunny weather the tops of the incubation chambers were covered with paper to keep the temperature from rising too high.

All seeds used in the following soil experiments were first moistened with 50 per cent alcohol and then immersed from two to four minutes in a 1:1000 solution of mercuric bichloride, after which they were rinsed in water. The soil and pots used were autoclaved from two to five hours. In some cases a spore suspension was added to the sterile soil, but in most cases the fungus was grown on autoclaved media and this was mixed with the steamed soil. Only the autoclaved medium was added to the check pots. The plants were kept fairly moist and their positions were interchanged from time to time in order to eliminate, as far as possible, environmental differences.

The following method was used in isolating the fungus: Seeds or pieces of the plant tissue from one to two centimeters long, were disinfected first by immersing them for half a minute in 50 per cent

ethyl or methyl alcohol and then in mercuric bichloride 1:1000 for from three to four minutes, after which they were rinsed in alcohol. The pieces were then plated out on potato dextrose agar in petri dishes. Isolations as a rule were made in duplicate.

Four-inch pots were used except when it was desired to grow plants to maturity, when eight-inch pots were used. The pots in which cereals were grown contained about twenty plants, but many more grass plants were grown in each pot.

The seeds of the grasses used were obtained from the grass gardens of the Bureau of Plant Industry at Lafayette, Ind., and Berkeley, Calif. Some were obtained from the Minnesota Seed Laboratory, and a few were collected locally. The seeds of corn and cereals were obtained from the Agronomy division, University of Minnesota, except the club wheat, which was obtained from the Washington State Agricultural Experiment Station.

## INOCULATION EXPERIMENTS

### HOST RANGE

The results of spraying cereals and grasses in the greenhouse and in the field with a spore suspension of *Helminthosporium sativum* are summarized in Table X. In all, 22 species and several varieties of wheat, oats, barley, rye, and corn, and 112 species of grasses were inoculated. All these species comprised 54 genera. Of the 134 species, 98 were successfully infected in the greenhouse. Fifty-eight were very susceptible, 40 were somewhat resistant, and the remaining 36 appeared to be immune.

Of the 66 species inoculated in the field, 57 became infected. All the species infected in the field were also infected in the greenhouse. Seven of the 9 species which were immune in the field did not become infected in the greenhouse, while the other 2 were slightly infected.

The degree of susceptibility or resistance was not based on the size or character of the lesions produced, but on the number of lesions and the effect of the fungus on the plant as a whole. When the plant was so severely attacked by the organism that numerous lesions were developed and the growth of the plant was either checked or inhibited, it was considered to be susceptible. Susceptible plants are indicated by the symbol H (heavy), while those less severely attacked are designated by M (medium). In cases where the presence of the fungus interfered only slightly with growth and development of the plant and only a few lesions were produced, the plant was considered to be resistant. Resistant plants are designated by L (light), Tr. (trace), and O (absence of infection). At times relatively small dead areas appeared as the only apparent indication of infection and the

symbol Fl. (flecks) was used to indicate this condition. Plus and minus signs are used to indicate slight variation within each class.

From Table X it will be noticed that the most susceptible cereals are wheat, barley, and rye, and that different species of oats and corn are practically all immune. The following common grasses are very susceptible: *Setaria viridis, Hystrix patula, Chloris verticillata* and various species of *Agropyron, Bromus, Elymus, Festuca, Hordeum,* and *Lolium.* The following are immune or extremely resistant: *Phleum pratense, Arrhenatherum elatius, Alopecurus pratensis, Koeleria cristata,* and species of *Phalaris, Poa,* and *Agrostis.*

The results shown in Table X establish the fact that there is a marked difference in the susceptibility of plants at different ages. For instance, *Bromus ciliatus* and *B. Kalmii,* inoculated when ninety-three days old, developed only a trace of infection, and eighteen days later similar plants were very susceptible. Numerous similar examples could be given, but in each case the resistance seems to be correlated with the stage of development of the tissue rather than with its actual age. Louise J. Stakman (9) called attention to similar results from inoculation experiments on wheat. Numerous field and greenhouse observations proved that plants are most susceptible to secondary infection after heading. In fact, spots on the stems and the characteristic blackening of the nodes seldom occur until after the plants are headed. Thus a plant may be immune or fairly resistant while small, but later prove extremely susceptible.

Typical lesions are developed on blades, sheaths, culms, glumes, seeds, and awns of cereals and grasses. Numerous inoculations and re-isolations of the fungus confirmed this conclusion. The organism was also repeatedly isolated from all the parts of naturally infected hosts mentioned above. This agrees with the statement made by Louise J. Stakman (9) that all parts of the host are susceptible.

### CROSS-INOCULATIONS

Several series of cross-inoculations with single spore strains of *Helminthosporium sativum* from wheat and barley produced the typical spot blotch on barley and, in general, gave results similar to those obtained with the strain isolated from rye. Helminthosporium strains obtained from Argentina, Canada, and Australia, not derived, however, from single spores, likewise caused typical spot blotch on barley and attacked the common wheats, durums, emmer, and rye in the same manner as did the barley strains.

TABLE X

RESULTS OF INOCULATING CEREALS AND GRASSES WITH *H. sativum* P., K., and B.

| Plants inoculated | Greenhouse inoculations* | | | | Field inoculations |
|---|---|---|---|---|---|
| | Series 1 | | Series 2 | | |
| | Age, days | Degree of infection† | Age, days | Degree of infection† | Degree of infection† |
| *Wheat* | | | | | |
| *Triticum compactum* | | | | | |
| Little Club C. I. 4066 | 46 | H | 62 | H | H |
| *Triticum dicoccum* | | | | | |
| Emmer C. I. 3686 | 46 | H | 104 | H | H+ |
| Khapli C. I. 4013 | 44 | L | 121 | H | H |
| *Triticum durum* | | | | | |
| Arnautka C. I. 4072 | 47 | L | 103 | L | M+ |
| Monad C. I. 3320 | 91 | M | 121 | M | H |
| *Triticum monococcum* | | | | | |
| Einkorn C. I. 2433 | 46 | H | 85 | H | H |
| *Triticum polonicum* | | | | | |
| Polish, Selection | 63 | H | 102 | H | ‡ |
| *Triticum spelta* | | | | | |
| Beardless spelt, Selection | 72 | M | 88 | M+ | M— |
| *Triticum vulgare* | | | | | |
| Kanred C. I. 5146 | 44 | L | 106 | H | M— |
| Marquis C. I. 3641 | 44 | H | 106 | H | L |
| *Oats* | | | | | |
| *Avena brevis* | | | | | |
| Selection | 66 | O | 73 | O | |
| *Avena fatua* | | | | | |
| Wild oats | 66 | O | 73 | O | |
| *Avena nuda* | | | | | |
| Selection | 66 | O | 73 | O | |
| *Avena sativa* | | | | | |
| Ligowa, Minn. No. 281 | 66 | O | 73 | O | |
| Victory, Minn. No. 514 | 99 | Tr | 130 | Fl | |
| *Avena sativa orientalis* | | | | | |
| Green Mountain, Selection | 66 | O | 73 | O | |
| *Avena sterilis* | | | | | |
| Red Rust Proof, Selection | 66 | O | 73 | O | |
| *Avena strigosa*, Selection | 66 | O | 73 | O | |
| *Barley* | | | | | |
| *Hordeum distichon palmella* | | | | | |
| Chevalier C. I. 278 | 32 | M— | 50 | H— | H— |
| Svanhals, Selection | 32 | L+ | 50 | H— | H+ |
| *Hordeum vulgare pallidum* | | | | | |
| Manchuria, Minn. No. 184 | 64 | L | 84 | H— | L+ |
| Bay Brewing, Selection | 44 | M— | 66 | H | H+ |
| *Hordeum vulgare trifurcatum* | | | | | |
| Nepal C. I. 262 | 64 | L | 68 | H— | H |
| *Rye* | | | | | |
| *Secale cereale* | | | | | |
| Swedish Rye, Minn. No. 2 | 31 | L | 110 | M+ | L— |
| Wisconsin Pedigree, Minn. No. 84 | 85 | Tr+ | 101 | L— | M— |

* The plants were sprayed with a suspension of spores.

† H=heavy infection; M=moderate infection; L=light infection; Tr=trace infection; O=no infection; Fl=flecks.

‡ A blank indicates that no plants were inoculated.

TABLE X--*Continued*

RESULTS OF INOCULATING CEREALS AND GRASSES WITH *H. sativum* P., K., and B.

| Plants inoculated | Greenhouse inoculations* | | | | Field inoculations |
|---|---|---|---|---|---|
| | Series 1 | | Series 2 | | |
| | Age, days | Degree of infection† | Age, days | Degree of infection† | Degree of infection† |
| *Corn* | | | | | |
| *Zea mays everta* | | | | | |
| White rice popcorn | 29 | O | 38 | Tr | |
| *Zea mays indurata* | | | | | |
| King Philip | 29 | O | 38 | Fl | |
| Longfellow | 29 | O | 38 | O | |
| *Zea mays indentata* | | | | | |
| Minn. No. 13 | 29 | O | 38 | O | |
| Rustler | 29 | O | 38 | O | |
| *Zea mays saccharata* | | | | | |
| Crosby | 29 | Fl | 38 | Tr | |
| Country Gentleman | 29 | Fl | 38 | Fl | |
| *Grasses* | | | | | |
| *Agropyron caninum* (L.) Beauv. | 104 | H | 105 | H+ | |
| *Agropyron cristatum* J. Gaert. | 104 | L | 152 | M+ | |
| *Agropyron disertorum* Schult. | 74 | L | 102 | H+ | |
| *Agropyron repens* (L.) Beauv. | 104 | M | 128 | M | |
| *Agropyron smithii* Rydb. | 104 | L | 128 | L | |
| *Agropyron spicatum* (Pursh.) Scribn. and Smith | 102 | H — | 156 | H | |
| *Agropyron tenerum* Vasey. | 40 | M | 55 | H+ | |
| *Agropyron tenerum longifolium* Scribn. and Smith | 40 | H | 105 | H | |
| *Agrostis alba* L. | 104 | O | 128 | O | O |
| *Agrostis palustris* Huds. | 102 | O | 156 | O | |
| *Agrostis stolonifera* L. | 87 | O | 115 | O | |
| *Aira caryophyllea* (L.) Nash. | 102 | O | 156 | O | |
| *Alopecurus pratensis* L. | 104 | Tr | 128 | Tr | L+ |
| *Andropogon sorghum* Brot. | 30 | L— | 128 | M | |
| *Andropogon sorghum sudanensis* Piper | 50 | L | 134 | L+ | |
| *Anthoxanthum odoratum* L. | 102 | L | 128 | L | M |
| *Arrhenatherum elatius* (L.) Beauv. | 104 | O | 126 | O | O |
| *Arundinaria* sp. Michx. | 30 | L— | 50 | Tr | |
| *Avena barbata* Brot. | 104 | O | 108 | O | |
| *Bechmania erucaeformis* (L.) Beauv. | 136 | Tr | | | Tr |
| *Briza media* L. | 104 | Tr | 118 | Tr | |
| *Bromus altissimus* Pursh. | 93 | Tr | 111 | M | H |
| *Bromus arvensis* L. | 93 | Tr | 111 | M | H |
| *Bromus brizaeformis* Fisch. and Mey. | 70 | Tr | 105 | L+ | M |
| *Bromus ciliatus* L. | 93 | Tr | 111 | H | H |
| *Bromus hordcaceus* L. | 33 | L | | | |
| *Bromus inermis* Leyss. | 144 | L | 156 | M | M |
| *Bromus japonicus* Thunb. | 93 | O | 111 | Tr | L |
| *Bromus kalmii* Grav | 93 | Tr | 111 | H | H |
| *Bromus langinosus* Poir. | 33 | L— | 102 | L+ | M |
| *Bromus marginatus* Nees. | 33 | Tr | 102 | L+ | H |
| *Bromus mollis* L. | 93 | Tr | 111 | L | M |
| *Bromus porteri* (Coult.) Nash. | 93 | O | 111 | M | H |
| *Bromus pumpellianus* Scribn. | 100 | Tr | 150 | L | H |

* The plants were sprayed with a suspension of spores.

† H=heavy infection; M=moderate infection; L=light infection; Tr=trace infection; O=no infection; Fl=flecks.

TABLE X—*Continued*

RESULTS OF INOCULATING CEREALS AND GRASSES WITH *H. sativum* P., K., and B.

| Plants inoculated | Greenhouse inoculations* | | | | Field inoculations |
|---|---|---|---|---|---|
| | Series 1 | | Series 2 | | |
| | Age, days | Degree of infection† | Age, days | Degree of infection† | Degree of infection† |
| *Grasses*—Continued | | | | | |
| *Bromus purgans* L. | 93 | Tr | 111 | H | H |
| *Bromus rubens* L. | 33 | L | 102 | L+ | L |
| *Bromus secalinus* L. | 93 | L | 111 | L | M |
| *Bromus sterilis* L. | 33 | M | 100 | L | H |
| *Bromus tectorum* L. | 104 | M | 128 | M | M |
| *Bromus unioloides* (Willd.) H.B.K. | 69 | L | 73 | L | H |
| *Bromus villosus* Forsh. | 69 | L | 102 | L | |
| *Capriola dactylon* (L.) Kuntze | 136 | L | 136 | M− | |
| *Cenchsus echinatus* L. | 55 | L− | 70 | M+ | Tr |
| *Chloris gayana* Kunth. | 70 | L− | 102 | L− | L− |
| *Chloris verticillata* Nutt. | 118 | H | 130 | H | |
| *Chloris virgata* Swartz. | 70 | M+ | | | L+ |
| *Cinna arundinacea* L. | 75 | O | 150 | O | |
| *Cynosurus cristatus* L. | 136 | O | 150 | O | |
| *Dactylis glomerata* L. | 125 | O | 143 | Tr | L− |
| *Danthonia intermedia* Vasey. | 90 | Tr | 102 | L | |
| *Deschampsia elongata* (Hook.) Munro | 102 | M | 152 | L+ | |
| *Deschampsia flexuosa* V. L. Trin. | 136 | O | 152 | O | |
| *Deyeuxia Forsteri* Kunth. | 77 | O | 87 | O | |
| *Digitaria humifusa* Pers. | 70 | O | 102 | O | |
| *Echinochloa crusgalli* (L.) Beauv. | 33 | Tr | 116 | L | L |
| *Echinochloa crusgalli zelayensis* Hitchc. | 33 | L− | 102 | L | M+ |
| *Elymus canadensis* L. | 63 | M− | | | |
| *Elymus robustus* Scribn. and J. J. Sm. | 86 | M+ | 136 | H− | |
| *Elymus virginicus* L. | 144 | M | 166 | H | H |
| *Eragrostis abyssinia* Schrad. | 75 | O | 136 | O | O |
| *Euchlaena mexicana* Schrad. | 30 | Tr+ | 42 | L+ | |
| *Festuca confinis* Vasey | 90 | L | 122 | H | L− |
| *Festuca dumetorum* Phil. | 104 | L | 122 | Tr | M |
| *Festuca elatior* L. | 73 | Tr+ | 122 | M | M+ |
| *Festuca myuros* L. | 33 | O | 105 | O | |
| *Festuca octoflora* Walt. | 122 | L | 156 | M− | |
| *Festuca ovina* L. | 104 | L | | | |
| *Festuca rubra* L. | 125 | Tr | 125 | Tr | O |
| *Gastridium lendigerum* (Gouan) Schinz and Thell. | 55 | O | 102 | O | |
| *Glyceria nervata* (Willd.) Trin. | 144 | O | 162 | Tr− | |
| *Holcus lanatus* L. | 104 | M | 162 | L | H |
| *Hordeum jubatum* L. | 104 | L | 117 | L− | M |
| *Hordeum murinum* L. | 144 | M | 117 | M | H |
| *Hordeum pusillum* Nutt. | 104 | L | 117 | M | |
| *Hystrix patula* Moench. | 104 | H | 117 | H | H |
| *Koeleria cristata* (L.) Pers. | 144 | O | | | O |
| *Lolium italicum* R. Br. | 87 | M | 90 | L | H |
| *Lolium perenne* L. | 104 | M | 122 | L | H |
| *Lolium subulatum* Vis. | 33 | L | 105 | L+ | H |
| *Lolium temulentum* L. | 102 | M− | 162 | M | H |

† H=heavy infection; M=moderate infection; L=light infection; Tr=trace infection; O=no infection; Fl=flecks.

TABLE X—*Continued*

RESULTS OF INOCULATING CEREALS AND GRASSES WITH *H. sativum* P., K., and B.

| Plants inoculated | Greenhouse inoculations* | | | | Field inoculations |
|---|---|---|---|---|---|
| | Series 1 | | Series 2 | | |
| | Age, days | Degree of infection† | Age, days | Degree of infection† | Degree of infection† |
| *Grasses*—Continued | | | | | |
| *Muhlenbergia racemosa* (Michx.) M.S.P. | 104 | L | 104 | M | |
| *Oryzopsis miliacea* Davy. | 102 | H | 156 | H+ | |
| *Panicum plicatum* Lam. | 75 | L+ | 156 | H+ | |
| *Panicum frumentaceum* Roxb. | 55 | Tr | 70 | L— | |
| *Pappohorum vaginatum* Buckl. | 100 | O | 120 | M | |
| *Papsalum setaceum* Michx. | 136 | O | 152 | O | |
| *Pennisetum villosum* R. Br. | 40 | Tr | 55 | L— | O |
| *Phalaris arundinacea* L. | 30 | O | | | L— |
| *Phalaris bulbosa* Tenore. | 87 | O | 102 | Tr+ | L— |
| *Phalaris caroliniana* Walt. | 102 | O | 156 | O | |
| *Phalaris minor* Retz. | 104 | O | 164 | O | |
| *Phleum pratense* L. | 105 | O | 111 | O | O |
| *Poa annua* L. | 55 | O | 156 | O | |
| *Poa brachyphylla* Schultes | 104 | O | 122 | O | |
| *Poa nemoralis* L. | 104 | O | 122 | O | |
| *Poa palustris* L. | 156 | O | 156 | O | |
| *Poa pratensis* L. | 104 | O | 122 | O | |
| *Poa triflora* Gilib. | 104 | O | 122 | O | O |
| *Poa trivalis* L. | 104 | O | 122 | O | |
| *Polypogon littoralis* J. E. Smith | 70 | O | 105 | O | |
| *Polypogon maritimus* Willd. | 156 | O | 156 | O | |
| *Polypogon monspeliensis* (L.) Desf. | 70 | L | 100 | Tr | |
| *Puccinella airoides* (Nutt.) Wats. and Coult. | 104 | M | 122 | L | |
| *Puccinella simplex* Scribn. | 136 | M— | 136 | M | |
| *Saccharum officinarum* L. | 30 | M | 104 | H— | M |
| *Setaria glauca* (L.) Beauv. | 33 | L | 70 | L | M |
| *Setaria viridis* (L.) Beauv. | 70 | H— | 104 | H | M |
| *Sphenopholis obtusata* (Michx.) Scribn. | 104 | O | 122 | O | O |
| *Sporobolus cryptandrus* (Tor.) Gray | 136 | Tr | 150 | Tr | |
| *Stipa* sp. L. | 33 | L | 87 | Tr | |
| *Triodia albescens* Vasey | 70 | O | 87 | Tr | |
| *Triticum aegilops* Beauv. | 33 | H+ | 105 | H+ | H+ |

† H=heavy infection; M=moderate infection; L=light infection; Tr=trace infection; O=no infection; Fl=flecks.

## SOIL INFECTION EXPERIMENTS

### ARTIFICIALLY INOCULATED SOIL

Seven varieties of wheat were inoculated, namely, Marquis, C. I. 3641; Kota, C. I. 5878; Kanred, C. I. 5146; Mindum, C. I. 5296; Acme, C. I. 5284; Monad, C. I. 3320; and Red Durum, Selection. The fungus was grown on autoclaved oatmeal, and both the fungus and the medium were mixed with the sterile soil. Uninoculated oatmeal was added to the checks.

The inoculated plants came up more slowly than those which had not been inoculated and the first leaf often developed typical leaf spots. Within a few days the browning of the basal sheath began to appear. The roots became brown and several of the plants died. The mortality among Marquis, Red Durum, and Kota plants was especially high. (See Plate VII, Fig. 2.) Mindum and Kanred appeared to be most resistant, while Acme was moderately susceptible. As some of the seeds used were already infected with Helminthosporium, a few of the check plants also became diseased.

In a similar but more extensive experiment, Marquis and Kanred wheats were used. Fifteen pots of each were inoculated as above, except that the fungus was grown on sterilized wheat instead of on oatmeal. In preparing this medium, dry wheat was barely covered with water and autoclaved for forty-five minutes. When the secondary roots were fairly well formed, the soil was carefully removed and the roots were washed clean in water. The root system of Kanred was usually about twice as extensive as that of Marquis. The roots of Kanred also were but slightly infected, while those of Marquis were usually quite heavily infected and broke off easily at the base of the stem.

On February 13, 1921, another series of seeds were inoculated like those in the previous experiment. In this case the fungus was grown on potato dextrose agar and this was added to the soil. The results are given in Table XI. The number of brown basal sheaths is rather low, as the notes were taken a few days too early to give the best results. Probably all the basal sheaths of the inoculated plants were brown at this time, but had as yet not made their appearance above the ground line, for a week later all the plants in the inoculated soil were characteristically discolored. There were lesions on the sheaths and roots of all the plants that were examined. The basal sheaths of the check plants of rye and wheat became somewhat discolored. This discoloration was undoubtedly due to physiological rather than to parasitic causes.

From Table XI it can readily be seen that the number of primary lesions on the leaves does not necessarily indicate the degree of susceptibility to root infection. For instance, primary lesions developed on six leaves of Red Durum and one developed on Monad, yet both are susceptible. The table also shows that the fungus caused a stunting of the plants. Twenty per cent of the inoculated rye plants were thus affected. Stunted plants form the second leaf below or at the ground line or a few millimeters above it. Typical lesions nearly always appear on the sheaths at or below the surface of the soil. The primary leaf was often rotted off completely at the ground line.

TABLE XI

RESULTS OF INOCULATING SEEDS OF WHEAT, BARLEY, RYE, AND OATS WITH *H. sativum*

| Variety | Number of plants | | Primary lesions on leaves | | Number of plants stunted | | Dark colored basal sheaths | |
|---|---|---|---|---|---|---|---|---|
| | Inoculated | Check | Inoculated | Check | Inoculated | Check | Inoculated | Check |
| *Wheat*—Kota, C. I. 5878.. | 23 | 23 | 2 | 0 | 0 | 0 | 10 | 0 |
| Acme, C. I. 5284..... | 29 | 29 | 5 | 0 | 0 | 0 | 17 | 2 |
| Mindum, C. I. 5296.... | 23 | 23 | 4 | 0 | 0 | 0 | 12 | 0 |
| Kanred, C. I. 5146..... | 30 | 4* | 7 | 0 | 1 | 0 | 11 | 0 |
| Monad, C. I. 3320..... | 8 | 8 | 1 | 0 | 0 | 0 | 8 | 1 |
| Marquis, C. I. 3641..... | 16 | 16 | 9 | 0 | 1 | 0 | 10 | 0 |
| Red Durum, Selection.. | 34 | 34 | 6 | 0 | 2 | 0 | 34 | 0 |
| Red Cross, Selection.... | 13 | 13 | 3 | 0 | 1 | 0 | † | |
| Red Wave, Selection.... | 18 | 18 | 2 | 0 | 1 | 0 | | |
| Illini Chief, Selection.... | 13 | 13 | 6 | 0 | 1 | 0 | | |
| *Barley*—Manchuria, Minn. No. 184 ..... | 16 | 16 | 6 | 0 | 0 | 0 | 16 | 0 |
| *Rye*—Minn. No. 2 ..... | 20 | 20 | 8 | 0 | 5 | 0 | 8 | 0 |
| *Oats*—Ligowa, Minn. No. 281 ..... | 2* | 8 | 0 | 0 | 0 | 0 | 0 | 0 |

* Some plants were eaten by rats.

† A blank indicates that notes were not taken.

Measurements were made of individual plants of one variety of barley, one of rye, and six of wheat. The data are summarized in Table XII. It will be noticed that on an average the inoculated plants produced the first leaf nearer the ground line than did normal plants.

TABLE XII

EFFECT OF HELMINTHOSPORIUM ON LOCATION OF THE FIRST LEAF*

| Red Cross | | Illini Chief | | Red Wave | | Monad | | Kanred | | Marquis | | Rye | | Barley | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Inoculated | Check | Inoculated | Check | Inoculated | Check | Inoculated | Check | Inoculated | Check | Inoculated | Check | Inoculated | Check | Inoculated | Check |
| cc. | cc. | cc. | cc. | cc. | cc. | cc. | cc. | cc. | cc. | cc. | cc. | cc. | cc. | cc. | cc. |
| 1.8 | 1.9 | 0.8 | 1.9 | 1.6 | 2.9 | 1.7 | 2.0 | 1.5 | 1.4 | 0.9 | 1.7 | 1.4 | 2.0 | 2.7 | 1.7 |
| 1.0 | 2.2 | 1.0 | 1.7 | 1.4 | 3.1 | 1.9 | 1.7 | 1.6 | 2.4 | 1.6 | 1.9 | 0.6 | 2.6 | 1.6 | 3.0 |
| 1.5 | 1.8 | 1.0 | 1.5 | 1.0 | 2.5 | 2.1 | 2.4 | 2.6 | 1.6 | 1.7 | 2.8 | 0.0 | 2.7 | 1.9 | 2.2 |
| 0.9 | 1.8 | 1.2 | 1.4 | 1.7 | 3.2 | 2.4 | 1.5 | 1.7 | 1.9 | 1.9 | 2.0 | 1.1 | 2.4 | 2.6 | 2.7 |
| 1.5 | 1.4 | 1.7 | 1.8 | 1.5 | 2.3 | 1.0 | 1.8 | 2.6 | 1.4 | 2.4 | 2.4 | 0.0 | 2.6 | 1.8 | 2.6 |
| 1.7 | 1.7 | 1.7 | 1.7 | 0.8 | 2.1 | 2.0 | 2.0 | 1.4 | 2.8 | 1.9 | 0.8 | 2.0 | 1.8 | 2.7 | 2.7 |
| 1.5 | 1.6 | 0.0 | 1.4 | 1.6 | 3.0 | 1.4 | 1.6 | † | ..... | 2.0 | 1.7 | 1.2 | 2.4 | 3.2 | 2.4 |
| 0.7 | 2.0 | 1.9 | 1.8 | 0.0 | 2.7 | 1.3 | 1.7 | ..... | ..... | 1.5 | 2.8 | 0.7 | 2.6 | 2.0 | 2.8 |
| 0.7 | 2.1 | 1.4 | 1.6 | 2.0 | 2.5 | ..... | ..... | ..... | ..... | 1.0 | 2.4 | 1.0 | 2.4 | 2.0 | 1.7 |
| 0.1 | 2.0 | 1.8 | 1.5 | 2.0 | 1.7 | ..... | ..... | ..... | ..... | 2.2 | 3.0 | 1.5 | 1.8 | 2.0 | 2.6 |
| 1.5 | 2.4 | 1.8 | 1.4 | 1.0 | 2.0 | ..... | ..... | ..... | ..... | 2.4 | 2.6 | 1.2 | 2.7 | 1.9 | 1.9 |
| 1.7 | 2.3 | 1.1 | 1.3 | 0.8 | 2.0 | ..... | ..... | ..... | ..... | 0.0 | 1.7 | 0.5 | 2.8 | 1.8 | 1.6 |
| 1.4 | 1.6 | 1.4 | 1.4 | 2.0 | 2.1 | ..... | ..... | ..... | ..... | 1.7 | 1.8 | 0.0 | 2.4 | 1.7 | 2.0 |
| † | ..... | 1.0 | 1.7 | 1.9 | 2.2 | ..... | ..... | ..... | ..... | 2.0 | 2.9 | 1.4 | 3.1 | 2.9 | 2.6 |
| ..... | ..... | 1.7 | 1.7 | 1.6 | 1.8 | ..... | ..... | ..... | ..... | 1.4 | 2.9 | 1.6 | 2.7 | 1.6 | 2.8 |
| ..... | ..... | 1.1 | 1.5 | 2.1 | 1.6 | ..... | ..... | ..... | ..... | 1.4 | 2.4 | 1.2 | 2.4 | 1.8 | 1.0 |
| Total ..... 16.0 | 24.8 | 20.6 | 25.3 | 23.0 | 37.7 | 13.8 | 14.7 | 11.4 | 11.5 | 26.0 | 35.8 | 15.4 | 39.4 | 34.2 | 36.3 |
| Average height... 1.28 | 1.90 | 1.28 | 1.51 | 1.43 | 2.35 | 1.72 | 1.83 | 1.90 | 1.91 | 1.62 | 2.23 | 0.96 | 2.46 | 2.13 | 2.26 |

* Distance of first leaf from ground line.

† A blank indicates that no plants were inoculated.

On healthy rye plants first leaves were produced about two and a half centimeters from the ground line, while on infected rye plants they usually appeared less than one centimeter above the ground. The position of the leaf on Marquis and Red Wave was affected greatly by the presence of the fungus. There was scarcely any effect on Kanred, however, and only slight effect on barley. This undoubtedly is due to the fact that Manchuria, Minn. No. 184, the barley used, is resistant to *Helminthosporium sativum* (4).

On April 20 the tops of the wheat plants grown in Experiment II were cut off and discarded. The roots, however, were mixed with the soil. The soil and pots of the check series were autoclaved for four or five hours because a few of the check plants were infected with *Helminthosporium*. The same soil was then resown with the same varieties of wheat, using twenty seeds in each pot. The pots were all placed on a center bench in the greenhouse.

From Table XIII it will be seen that the greatest number of primary lesions appeared on Acme, Monad, and Kota. All plants grown in unsterilized soil developed brown basal sheaths, and nearly all were spindling and considerably shorter than the checks. The checks, with the exception of three plants, remained free from infection.

TABLE XIII

Result of Planting Wheat Seeds in Infected Soil

| Variety planted* | Variety planted† | No. of plants | | Primary lesions on first leaf | | No. of plants stunted | |
|---|---|---|---|---|---|---|---|
| | | Inoculated | Check | Inoculated | Check | Inoculated | Check |
| Monad C. I. 3320 | Kota C. I. 2151 | 18 | 19 | 3 | 0 | 4 | 0 |
| Marquis C. I. 3641 | Monad C. I. 2156 | 18 | 19 | 2 | 0 | 7 | 0 |
| Red Durum Selection | Marquis C. I. 1239 | 17 | 19 | 1 | 0 | 1 | 0 |
| Kota C. I. 5878 | Kanred C. I. 5146 | 10 | 17 | 2 | 0 | 2 | 0 |
| Kanred C. I. 5146 | Mindum Minn. No. 470 | 20 | 20 | 1 | 0 | 2 | 0 |
| Acme C. I. 5284 | Red Durum C. I. 1446 | 14 | 20 | 0 | 0 | 2 | 0 |
| Mindum C. I. 5296 | Acme C. I. 1967 | 18 | 20 | 1 | 0 | 9 | 0 |

* Varieties of wheat first grown. Planted January 13, 1921.

† Succeeding varieties of wheat grown on the same soil and in corresponding pots. Planted March 20, 1921. Data taken from later planting.

Tables XIV and XV show the striking differences in height between plants grown in infected soil and those grown on autoclaved soil. (See Plate VII, Fig. 1.) Table XIV summarizes the height of plants eleven days after planting. Measurements were made from the ground line to the tip of the first leaf.

Kota was the most dwarfed of the seven varieties. Here the check plants were more than twice as high as the infected plants. Mindum appeared to be the most healthy, altho there was still a marked difference in size between the check and the infected plants.

Table XV gives the height of the plants eight days later. Measurements were made from the ground line to the tip of the highest leaf. It will be seen by referring to Tables XIV and XV that the effect of the fungus became more pronounced as the plants grew older. Marquis, however, recovered somewhat.

The appearance of the infected plants was similar to that described in the previous experiments. Subsequently the plants were grown almost to maturity. The striking difference between the check and the infected plants not only continued to exist, but became even more marked as the plants began to head. (See Plate VIII.)

In order to determine whether the fungus would attack the roots of grasses also, the following experiment was carried out: Eight four-inch pots of quartz sand were autoclaved for three hours under fifteen pounds pressure. The fungus was grown on sterilized wheat and this was mixed with the sand in four of the pots. To the control pots the same amount of steamed wheat was added, without the fungus. One check pot and one inoculated pot were sown with each of the following grasses: *Agropyron tenerum, Hordeum murinum, Bromus unioloides,* and *Lolium temulentum.* About the same number of seeds was used in the two pots for each species. Knop's nutrient solution was applied to all the pots after the seedlings were one or two inches high.

The results were very striking. The plants of all species were less vigorous in the inoculated pots than in the control pots. Nearly all the seedlings of *Agropyron tenerum* were killed (Plate III, Fig. 2.) *Lolium temulentum* was most resistant. Damping off was observed several times in the greenhouse on uninoculated plants of *Agropyron tenerum, A. caninum, Elymus canadensis, Hystrix patula,* and several others. Whenever cultures were made from such plants, *Helminthosporium sativum* was isolated.

TABLE XIV

EFFECT OF GROWING WHEAT IN INFECTED SOIL ON THE HEIGHT OF THE PLANT*

| | Kota | | Monad | | Marquis | | Kanred | | Mindum | | Red Durum | | Acme | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Inoculated | Check | Inoculated | Check | Inoculated | Check | Inoculated | Check | Inoculated | Check | Inoculated | Check | Inoculated | Check |
| | cc. | cc. | cc. | cc. | cc. | cc. | cc. | cc. | cc. | cc. | cc. | cc. | cc. | cc. |
| | 4.0 | 12.0 | 8.7 | ..... | 10.5 | 11.9 | 5.6 | 11.3 | 11.0 | 15.0 | 9.8 | 11.0 | 7.0 | 10.0 |
| | 6.5 | 11.8 | 7.5 | 13.0 | 9.2 | 12.5 | 3.9 | 8.8 | 11.8 | 12.2 | 4.0 | 10.5 | 7.7 | 13.0 |
| | 6.0 | 12.1 | 8.5 | 12.0 | 11.5 | 12.7 | 7.8 | 11.2 | 12.9 | 14.7 | 11.1 | 11.4 | 8.5 | 11.7 |
| | 7.2 | 12.5 | 11.5 | 12.5 | 8.7 | 12.5 | 7.5 | 7.7 | 7.6 | 13.6 | 10.6 | 13.5 | 10.5 | 11.4 |
| | 6.0 | 12.2 | 5.7 | 12.0 | 7.5 | 12.5 | 8.5 | 6.4 | 11.0 | 13.8 | 8.5 | 5.4 | 6.0 | 12.5 |
| | 7.5 | 11.9 | 8.7 | 14.0 | 5.0 | 12.0 | 6.8 | 8.8 | 12.0 | 15.0 | 10.1 | 11.5 | 8.9 | 14.1 |
| | 3.5 | 13.0 | 7.0 | 11.5 | 11.0 | 12.6 | 2.2 | 7.4 | 10.5 | 14.6 | 11.0 | 9.4 | 9.0 | 12.2 |
| | 6.0 | 11.9 | 6.4 | 12.5 | 11.0 | 12.9 | 4.7 | 10.4 | 8.5 | 14.2 | 12.0 | 11.4 | 8.0 | 12.5 |
| | 11.0 | 12.4 | 8.0 | 11.5 | 11.0 | 11.8 | 3.5 | 8.7 | 9.5 | 13.8 | 12.5 | 13.2 | 8.6 | 11.8 |
| | 6.0 | 9.0 | 8.5 | 11.5 | 9.0 | 13.2 | 9.2 | 9.8 | 10.7 | 14.7 | 6.5 | 14.9 | 8.6 | 12.2 |
| | 6.0 | 12.5 | 8.7 | 13.7 | 6.5 | 12.8 | † | 9.5 | 10.5 | 13.0 | 11.5 | 11.6 | 9.8 | 14.7 |
| | 5.5 | 12.0 | 7.2 | 11.7 | 7.5 | 11.9 | ..... | 9.0 | 3.6 | 13.3 | 10.8 | 10.6 | 9.2 | 12.4 |
| | 5.4 | 12.7 | 8.0 | 9.0 | 10.0 | 13.5 | ..... | 10.9 | 10.1 | 13.5 | 9.7 | 13.5 | 8.0 | 11.0 |
| | 7.6 | 13.5 | 9.0 | 12.8 | 8.0 | 13.4 | ..... | 4.5 | 11.4 | 14.6 | 11.9 | 12.4 | 9.6 | 11.3 |
| | 11.2 | 11.2 | 4.3 | 11.1 | 12.5 | 12.0 | ..... | 5.5 | 10.7 | 15.2 | ..... | 10.5 | 5.5 | 14.1 |
| | 6.5 | 12.8 | 11.0 | 12.7 | 10.5 | 13.2 | ..... | 8.0 | 11.2 | 13.4 | ..... | 7.5 | 7.9 | 12.9 |
| | 11.0 | 13.4 | 8.2 | 12.4 | 9.0 | 13.0 | ..... | 10.0 | 12.7 | 12.5 | ..... | 8.6 | 10.3 | 12.5 |
| | 6.5 | 12.7 | 6.2 | 12.5 | ..... | ..... | ..... | ..... | 8.7 | 14.7 | ..... | 11.0 | 9.5 | 10.9 |
| | ..... | 12.0 | ..... | 13.0 | ..... | ..... | ..... | ..... | 11.6 | 14.7 | ..... | 13.5 | ..... | 8.2 |
| | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... | 11.9 | 15.1 | ..... | 9.5 | ..... | 12.3 |
| Total .. | 124.0 | 232.7 | 143.1 | 219.5 | 159.3 | 214.4 | 59.4 | 147.9 | 208.0 | 281.6 | 140.0 | 220.9 | 152.6 | 241.7 |
| Average | 6.88 | 12.27 | 7.95 | 12.19 | 9.37 | 12.61 | 5.94 | 8.70 | 10.40 | 14.08 | 10.0 | 11.04 | 8.47 | 12.08 |

* Measurements made eleven days after planting. For height eight days later see Table XV.

† A blank indicates that no plants were inoculated.

TABLE XV

EFFECT ON HEIGHT OF PLANT OF GROWING WHEAT IN INFECTED SOIL*

| | Kota | | Monad | | Marquis | | Kanred | | Mindum | | Red Durum | | Acme | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Inoculated | Check | Inoculated | Check | Inoculated | Check | Inoculated | Check | Inoculated | Check | Inoculated | Check | Inoculated | Check |
| | cc. | cc. | cc. | cc. | cc. | cc. | cc. | cc. | cc. | cc. | cc. | cc. | cc. | cc. |
| | 21.4 | 35.4 | 21.6 | 32.0 | 14.5 | 25.6 | 9.0 | 28.2 | 4.2 | 30.5 | 17.6 | 30.3 | 8.2 | 28.7 |
| | 19.9 | 31.8 | 10.4 | 30.3 | 14.7 | 27.1 | 12.2 | 22.0 | 8.0 | 31.4 | 11.0 | 16.7 | 14.3 | 17.8 |
| | 23.5 | 24.5 | 17.9 | 26.7 | 13.5 | 28.8 | 9.4 | 23.8 | 24.0 | 30.2 | 18.1 | 23.9 | 13.5 | 24.2 |
| | 13.4 | 32.0 | 10.0 | 26.8 | 9.0 | 24.2 | 16.0 | 17.4 | 24.6 | 26.7 | 15.4 | 22.5 | 17.9 | 29.2 |
| | 13.6 | 32.4 | 6.4 | 30.6 | 16.5 | 27.4 | 12.8 | 26.9 | 14.4 | 25.4 | 19.6 | 24.0 | 10.6 | 21.6 |
| | 12.2 | 36.2 | 13.2 | 21.6 | 12.6 | 25.5 | 15.7 | 27.5 | 15.4 | 22.0 | 19.7 | 24.4 | 11.9 | 30.6 |
| | 10.5 | 32.6 | 14.6 | 24.0 | 14.4 | 26.5 | 19.0 | 26.5 | 23.7 | 29.4 | 16.0 | 25.2 | 8.6 | 35.8 |
| | 18.1 | 31.1 | 15.5 | 25.7 | 16.7 | 25.4 | 14.4 | 28.1 | 15.5 | 30.4 | 14.5 | 24.1 | 14.6 | 31.0 |
| | 11.2 | 30.2 | 13.7 | 28.4 | 18.0 | 24.2 | 17.3 | 29.4 | 17.8 | 32.3 | 19.6 | 24.1 | 15.2 | 22.5 |
| | 12.0 | 23.0 | 14.4 | 19.4 | 15.5 | 26.3 | 9.6 | 20.4 | 15.8 | 30.2 | 18.5 | 23.9 | 13.1 | 30.3 |
| | 15.1 | 33.0 | 9.7 | 25.0 | 16.3 | 26.9 | 14.7 | 15.8 | 17.0 | 24.5 | 18.0 | 24.2 | 14.2 | 31.4 |
| | 9.0 | 35.0 | 20.3 | 29.0 | 17.6 | 26.5 | 17.9 | 23.0 | 11.7 | 26.4 | 17.0 | 22.6 | 9.5 | 24.9 |
| | 9.5 | 35.2 | 14.6 | 22.6 | 15.0 | 24.2 | † | 18.3 | 23.0 | 23.5 | 16.1 | 20.6 | 7.0 | 28.0 |
| | 9.6 | 34.8 | 12.4 | 34.0 | 18.0 | 28.9 | ..... | 20.0 | 22.8 | 31.2 | ..... | 29.0 | 9.6 | 25.6 |
| | 10.9 | 28.5 | 14.5 | 26.9 | 16.4 | 25.4 | ..... | 22.2 | 21.7 | 25.0 | ..... | 23.6 | 15.3 | 21.5 |
| | 14.4 | 23.0 | 12.3 | 28.5 | 14.7 | 26.1 | ..... | 16.5 | 25.4 | 28.3 | ..... | 25.0 | 14.6 | 29.2 |
| | 19.5 | 32.0 | 15.6 | 32.8 | 16.5 | 23.5 | ..... | 15.6 | 21.4 | 33.2 | ..... | 23.7 | 15.8 | 23.8 |
| | 17.3 | 31.0 | 21.2 | 19.1 | ..... | ..... | ..... | ..... | 21.5 | 29.0 | ..... | 26.5 | 14.6 | 25.7 |
| | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... | 22.0 | 24.1 | ..... | 31.9 | ..... | 35.9 |
| | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... | ..... | 29.5 | ..... | ..... | ..... | 32.3 |
| Total .. | 261.1 | 561.7 | 258.3 | 483.4 | 259.9 | 442.5 | 168.0 | 381.6 | 349.9 | 563.2 | 221.1 | 466.2 | 228.5 | 550.0 |
| Average | 12.5 | 31.1 | 14.4 | 26.8 | 15.2 | 26.2 | 14.0 | 22.4 | 18.4 | 28.1 | 17.0 | 24.5 | 12.8 | 27.5 |

* Measurements made nineteen days after planting. For height eight days earlier see Table XIV.

† A blank indicates that no plants were inoculated.

NATURALLY INFESTED SOIL

In order to determine whether root rot and stunting of susceptible varieties of wheat and barley would occur when clean seed was sown in naturally infested soil, the following experiments were carried out in the greenhouse during the winter of 1921-22. Soil was taken from a field which had grown barley in 1920 and 1921, and which previous to that time had produced truck crops and grasses. In both of the years in which barley was grown, the plants were sprayed with a suspension of spores, and in 1921 were severely infected with root-rot. The soil was placed in twenty eight-inch pots. Ten of these were autoclaved for three hours under a pressure of fifteen pounds.

The following varieties were used: Manchuria barley, Minn. No. 184, and Arequipa selection; Marquis wheat, C. I. 3641; Monad durum, C. I. 3320; and Victory oats, Minn. No. 514. Fifteen seeds which had been disinfected in the regular manner were sown in each pot. Two series were carried out, in each of which one pot of sterilized and one of unsterilized soil were used for each of the five varieties. The positions of the pots were interchanged every seven to ten days. The final notes on the comparative development of the plants were taken when they were beginning to head. Measurements were made from the ground line to the tip of the highest leaf. Table XVI summarizes the data obtained from this experiment. In both series Marquis and Monad wheat and Arequipa barley in unsterilized soil became very much stunted. For instance, the check plants of Marquis in Series 1 were, on the average, more than twenty-nine centimeters higher than those in non-autoclaved soil. Table XVI shows this marked difference. Manchuria barley, which is resistant in the field, appeared resistant also in this experiment, altho the height of the check plants should be greater than expressed in the table, as three or four plants in the check pots became severely infected from seed infections and did not develop normally. The average differences in the two series of oats are insignificant. The infected plants of Marquis produced on the average two or three culms, while the control plants developed only one. This experiment and field observations of the plants grown in the plots from which this soil was taken, indicate that soil harbors the pathogene. The facts that Manchuria grew about equally well on sterilized and non-sterilized soil and that oats grew slightly better in the control pots, indicate that the effects noted were not due to toxins. Moreover, the soil had not grown cereals for ten years and it is not probable that barley could excrete enough toxins in one year to stunt the plants. *Helminthosporium sativum* is apparently an important agent in making soils unproductive.

TABLE XVI

EFFECT OF GROWING WHEAT, BARLEY, AND OATS ON NATURALLY INFECTED SOIL

| Variety | Series 1 | | | | Series 2 | | | | Average difference in height of plants | |
|---|---|---|---|---|---|---|---|---|---|---|
| | Number of plants | | Average height of plants | | Number of plants | | Average height of plants | | | |
| | Infected soil | Check | Infected soil | check | Infected soil | Check | Infected soil | Check | Series 1 | Series 2 |
| | | | c.m. | c.m. | | | c.m. | c.m. | c.m. | c.m. |
| *Wheat* | | | | | | | | | | |
| Marquis, C. I. 3641 | 10 | 11 | 46.60 | 76.18 | 10 | 11 | 52.70 | 76.54 | +29.58* | +23.84 |
| Monad, C. I. 3320 | 12 | 12 | 51.41 | 71.41 | 13 | 12 | 65.84 | 72.08 | +20.00 | +6.24 |
| *Barley* | | | | | | | | | | |
| Arequipa Selection | 11 | 13 | 48.63 | 57.61 | 11 | 15 | 50.72 | 57.73 | +8.98 | +7.01 |
| Manchuria, Minn. No. 184 | 13 | 14 | 50.07 | 54.25 | 10 | 15 | 59.40 | 55.46 | +4.18 | −3.94 |
| *Oats* | | | | | | | | | | |
| Victory, Minn. No. 514 | 14 | 11 | 66.27 | 63.85 | 13 | 13 | 78.61 | 81.07 | −2.42 | +2.36 |

* The plus sign indicates an increase and the minus sign a decrease in the height of the check plants.

INFECTION RESULTING FROM DISEASED SEED

An equal number of black-tipped and badly discolored seeds of wheat and barley were disinfected and planted in sterilized quartz sand. Duplicate pots were planted. Most of the wheat and barley plants developed from diseased seeds were much less vigorous than those produced from clean seeds. When the seedlings were about two weeks old the sand was carefully washed away from the roots. It was found that the fungus was fruiting luxuriantly on many of the infected seeds. Some of the seeds had been attacked so rapidly and so severely that they failed to germinate. Others had germinated but the shoots were killed before reaching the surface. Both roots and stems were sometimes destroyed. (See Plate II.) Many of the plants that survived were stunted and weak. There were brown lesions on the roots and foot, and on the basal sheaths of the plants developed from the infected seeds. Usually the roots just below the crown were most severely infected. The length of the diseased areas varied from a fraction of a centimeter to three centimeters or more. The difference in root development also was very marked. The roots were not nearly so numerous or so vigorous as those on plants from healthy seed. This experiment was repeated and very similar results were obtained. Infected seeds of *Hordeum murinum* were sown in sterile sand and the results were similar to those described for wheat and barley.

The fungus was isolated repeatedly from seeds of many varieties of wheat, barley, and rye, and from several species of grasses. Samples of Monad wheat from North Dakota showed almost 100 per cent infection when the seeds were plated out on agar. From 50 to 75 per cent of infected seeds is not uncommon for many varieties of wheat and barley at University Farm.

Louise J. Stakman (9) found that when infected seeds were sown in the field they germinated poorly, the seedlings were weak and spindling, and there was a characteristic foot-rot and distinct rosetting due to excessive stooling.

## VITALITY OF THE FUNGUS

The conditions under which Helminthosporium overwinters have been imperfectly known. Investigations were undertaken to find out whether the mycelium and the spores both overwinter. In the fall of 1921, infected wheat and barley plants were left unprotected in the field, and some were stored in a dry basement. Later in the fall several bottles containing agar or sterilized wheat straw were inoculated with Form I of *Helminthosporium sativum*. When the substratum was fairly well covered with spores, they were treated in the following

manner. Two bottles containing agar and two containing wheat straw were covered with from five to seven centimeters of water; four similar cultures were covered with five to ten centimeters of sand. In each case one set was placed outdoors in the fall of 1921 and the other was kept in the laboratory at room temperature. Isolations were made on April 16, 1922. The results given in Table XVII indicate that the fungus does overwinter in the field unprotected and also when imbedded in ice or covered with moist sand.

Hanging drop cultures were made from spores kept outside over winter on agar and covered with moist sand or water. No germination was obtained. These experiments indicate that spores do not overwinter under moist conditions, but that the mycelium survives.

In the laboratory the spores remained viable for a long period. Thus spores kept covered with moist sand for more than five months germinated readily. Likewise spores from a transfer twenty-three months old kept at ordinary room temperature germinated when placed in drops of water.

The data in Table XVII show that the fungus retains its viability in the laboratory under varying conditions for a considerable length of time. Numerous plantings from diseased seed kept indoors proved that the mycelium may live for several years; in fact, the organism was isolated during the spring of 1921 from seed of the 1914 crop. Usually, however, the hyphae in such seed are dead. An unsuccessful attempt also was made to isolate the fungus from badly diseased leaves which had been kept in the laboratory since 1915, i.e., for a period of six and a half years.

TABLE XVII

RESULTS OF OVERWINTERING STUDIES ON *Helminthosporium sativum* 1921–1922

| Substratum | Treatment | Date begun | Results* | |
|---|---|---|---|---|
| | | | Outside† | Laboratory |
| Potato dextrose agar | Covered with from 5 to 10 centimeters of moist sand | 11-14-21 | Positive | Positive |
| Potato dextrose agar | Covered with from 5 to 7 centimeters of water | 11-14-21 | Positive | Positive |
| Wheat straw | Covered with from 5 to 10 centimeters of moist sand | 12- 2-21 | Positive | Positive |
| Wheat straw | Covered with from 5 to 7 centimeters of water | 12- 2-21 | Positive | Positive |
| Wheat straw | Covered with from 5 to 10 centimeters of black loam | 12- 2-21 | Positive | ? |
| Wheat straw, roots, and glumes | Left in field and stored in basement | Fall, 1921 | Positive | Positive |
| Barley straw, kernels, and awns | Left in field and stored in basement | Fall, 1921 | Positive | Positive |

* Cultured April 16, 1922.

† Part of each set was placed in the laboratory and part outside.

## SOURCE OF INFECTION

The initial infections in the field and greenhouse come from seed or from soil. The fungus, as already indicated, was isolated more than 200 times from discolored seeds of more than one hundred species and varieties of wheat, barley, rye, and grasses. Many seeds of barley and durum wheat were planted on agar and colonies of Helminthosporium developed on from 85 to 100 per cent of the seeds of some varieties. When black-pointed seed of barley and wheat were germinated on sterile blotting paper or planted in sterilized quartz sand, Helminthosporium often grew from them. Miss Evans (2) obtained similar results with wheat.

Plants grown from such discolored seed nearly always produce a foot- and root-rot from which *Helminthosporium sativum* was repeatedly isolated, while plants grown from clean seeds remained healthy. Waterhouse (11) observed the hyphae of Helminthosporium in young plantlets of wheat, the infection having resulted from diseased seed. The disease was reproduced in the greenhouse from soil obtained from infected areas in the field. Stevens (10) obtained similar results.

The above experiments on overwintering demonstrate that the fungus overwinters in the field on old straw, roots, and seed. In fields of wheat which had been severely infected with seedling blight and root-rot in 1919, the disease was again destructive in 1921. It is hard to correlate definitely the seriousness of the disease with crop history, because the amount of injury depends also on other factors, such as temperature, moisture, etc.

Secondary infection results from a variety of sources. In the spring the fungus has been observed to fruit on the nodes of plants of the previous season. The fungus also sporulates freely on primary lesions. The spores are then distributed by such agents as rain, water, and wind, the last undoubtedly being the chief factor. In the recent rust epidemiology studies made by the Office of Cereal Investigations of the United States Department of Agriculture (8) during the spring and summer of 1921, numerous spores of Helminthosporium were caught by spore traps at elevations up to ten thousand feet.

Once the fungus has sporulated on the plants, repeated inoculations and infection may occur as in cereal rusts, potato late blight, and similar diseases. It was found by experimentation that the mycelium and spores remained alive in the laboratory after having been buried in coarse moist sand for more than five months. The longevity of the spores and mycelium probably plays an important part in producing an epidemic of this disease.

The effect of wild grasses on increasing secondary infections of cereals is important, as a large number of the common grasses are susceptible; and as the fungus overwinters on their remains they very likely aid greatly in the dissemination of the disease.

## CONTROL

The problem of controlling the disease has not been sufficiently investigated, and the control measures recommended at the present time are altogether inadequate. F. Kolpin Ravn (7) demonstrated clearly that Jensen's modified hot water treatment eliminated *Helminthosporium teres* and *H. gramineum* from the seeds. Louise J. Stakman (9) states that long-time soaking in formaldehyde reduced the amount of *Helminthosporium sativum.* These treatments, however, do not eliminate secondary or soil infections, and these are probably the most important. The following control measures suggest themselves in the light of the facts brought out in this investigation: (1) Planting clean seed; (2) Good cultural methods; (3) Rotation of crops; (4) Use of existing resistant varieties and the development of additional resistant varieties with more desirable agronomic characters.

Since it has been shown definitely that the disease is seed-borne and that infected seeds often produce diseased plants, clean seed is essential. That sowing grain late in the spring favors the development of root-rots is indicated by the fact that the optimum temperature for the pathogene is relatively high. It does not thrive nearly so well when the soil is cool (40° to 60° F.), whereas wheat, barley, and rye seedlings develop a better root system and are more vigorous at these temperatures than at the higher temperatures which are more favorable for the rapid growth of the fungus. Therefore if susceptible grains are grown they should be sown as early as possible in the spring. Observations at University Farm indicate that root-rots and seedling blight are more prevalent in fields which have been cropped continuously to wheat than in those on which rotation has been practiced. As the disease lives over in the soil or on the remains of susceptible plants, a rotation which includes an immune or highly resistant crop, such as corn, clover, timothy, or vegetables is beneficial in reducing the disease. Altho good cultural methods and the use of clean seed may reduce the amount of damage and in certain years may control the disease, the most promising method of control is the development of resistant varieties. Manchuria barley, Minn. No. 184, was resistant to several forms of *Helminthosporium sativum* in Minnesota. Coöperative breeding work between the Division of Plant Pathology and Botany and the plant breeding section has been under

way for some time at University Farm (4). Preliminary varietal tests were made with fifty varieties of wheat and they were partially checked in the greenhouse under controlled conditions. The results indicate that resistant varieties may be obtained. For example, Kanred wheat appears to be highly resistant to root-rot caused by Form I, while Marquis is susceptible to root-rot caused by this biologic form, but is fairly resistant to seed blight and head blight.

More detailed studies of the factors that favor the development and spread of the disease, the longevity of the fungus in the soil, as well as the number of biologic forms which occur, must be carried on to insure the best results in selecting and breeding resistant varieties.

## DISCUSSION AND CONCLUSIONS

It has been known for some time that *Helminthosporium sativum* caused the spot-blotch of barley, but it has not been known until recently that the same fungus also can cause root- and foot-rots, discoloration of the nodes, leaf spots, glume spots, and discoloration and shriveling of the kernels of the common small grains and a great many wild grasses.

Since Bolley first called attention to the destructiveness of the Fungi Imperfecti to cereals, considerable work has been done in attempting to ascertain the exact part played by certain of them in reducing yields of small grains. There seems to be no question whatever but that *H. sativum* is one of the most destructive of these fungi.

The losses caused by *H. sativum* are undoubtedly considerable. It appears that in Minnesota, at least, this organism is the cause of most of the root-rotting of wheat, barley, and rye. In some years the disease is very destructive. At times the damage is not conspicuous since only isolated individuals are killed, but at other times the plants are stunted or killed in definite, more or less circular areas, which are very striking. In either case, the aggregate damage may be considerable. Experimental work done at University Farm indicates clearly that losses may be very great when the soil is constantly cropped to susceptible varieties of cereals.

Not only is *H. sativum* a virulent parasite in various parts of the spring wheat region, but it apparently is quite widely distributed in the United States, having been isolated from grains and grasses from widely separated regions in this country. It is also known to occur in various regions of Canada, in Mexico, in the Argentine, and in Australia. Apparently it is not yet known in Europe. It is quite possible, however, that it occurs but that the damage which it does is confused with the damage caused by various other root- and foot-rotting fungi.

It has generally been considered that *Helminthosporium sativum* is a group species. Several investigators have noticed that the fungus varies considerably not only in cultural characters on different media but in spore size. This can be explained partially at least by the existence of several biologic forms which differ from each other very markedly in cultural characters. The morphology of the spores of the different forms varies slightly and there are some differences in their parasitic behavior. However, the principal difference is in their cultural characters. Each of these biologic forms is constant when grown under the same environmental conditions, but cultural characters and morphology of spores are profoundly influenced by changes in environmental conditions. The effect of the medium on which a form is grown, and the effect of temperature is sometimes so great as to make it practically impossible to distinguish the form. The consistent difference between different forms under known conditions and extreme variability of each individual form are very significant. This variability simply emphasizes the fact that extreme caution is necessary in determining species of Helminthosporium and other similar fungi which may vary quite as much as does this one. Descriptions of such organisms can be given only after careful studies have been made under known environmental conditions.

There are some indications that there are still more forms than those which have been discovered so far. It is quite possible that they may differ from each other pathogenically more sharply than do those now known. If this should be true, then the existence of these forms will complicate the problem of procuring varieties of cereals resistant to this organism.

The pathogenicity of *Helminthosporium sativum* was studied extensively. The host range is extremely wide. Not only does the organism attack wheat, barley, and rye but it also attacks about eighty-three species of wild grasses belonging to 37 different genera. This was demonstrated by making isolations from a great many wild grasses, by inoculation experiments in the field, and by carefully controlled inoculation experiments in the greenhouse. The fact that the organism can attack so many different hosts, under such wide range of conditions, makes its control extremely difficult.

The pathogene overwinters very readily in Minnesota in soil, in seed, and in dead-plant parts. It has been shown that hyphae may remain in seeds for several years. Since the organism also is a faculative saphrophyte and can grow on dead or dying plant remains and even in the soil, the number of spores is very large. A very high percentage of wheat seed is likely to be infected with the organism. Many primary infections therefore probably come from seed. It

has been shown, however, that the use of clean seed in infested soil does not protect plants from infection. Many experiments were made which indicate that primary infections may result from the spores or mycelium in the soil. Secondary infections are very numerous. They are caused principally by wind-blown spores. That the spores are very numerous and widespread is indicated by the fact that they have been caught by spore-traps in airplanes at altitudes up to 10,000 feet.

It is quite obvious that control measures are difficult. The use of clean seed, when it can be procured, is of course recommended. Furthermore, the seed should be sown on land which has not grown an infected crop for several years. The hyphae inside the seed are not killed by the ordinary treatment such as is given for the prevention of covered smuts. It is sometimes difficult to kill the intraseminal mycelium even by special methods. Soil can be kept reasonably free from the pathogene by practicing a proper system of rotation. The use of corn, potatoes, clover, or oats in the rotation would be highly beneficial, since none of these crops is susceptible to the disease. However, the wild weed grasses are practically always present. If our cultural practices included killing these grasses, it would assist greatly in controlling two of our very serious diseases, namely, stem rust and the Helminthosporium disease. Even tho clean seed is available and is sown on reasonably clean soil, there is always the danger of infection by means of wind-blown spores which have been developed on susceptible wild grasses. The most promising method of control is the development of resistant varieties. Considerable progress already has been made in the development of resistant types of barley, and there are indications that there are sufficient differences in the susceptibility of varieties of wheat to make it possible to select or breed desirable resistant varieties.

## SUMMARY

1. *Helminthosporium sativum* Pammel, King, and Bakke, which causes a serious disease of wheat, barley, and rye in Minnesota, appears to be very widely distributed, since inoculations with cultures obtained from different regions of the United States, Canada, Argentina, and Australia produced typical spot blotch on barley. The same organism was also isolated from wheat grown in Mexico.

2. *H. sativum* causes leaf spots, root-rots, foot-rots, seedling blight, head blight, and badly discolored seeds of wheat, barley, and rye, and many grasses. The organism also causes stunting of wheat, barley, and rye and excessive stooling of wheat and barley.

3. No parts of the susceptible plant are immune. Roots, stems, leaves, glumes, awns, and seeds may be attacked. Plants may be highly resistant, or even immune in the early stage of development, but later the same plants may become very susceptible.

4. Hundreds of isolations were made from infected plants found not only in Minnesota and adjoining states but also in various regions of the United States and other countries. The fungus was isolated from many naturally infected cereals and grasses, including several varieties of rye, 20 varieties of barley, 70 varieties of wheat, and 32 species of common grasses.

5. Biologic specialization occurs in *Helminthosporium sativum*. At least four forms have been found. These forms differ physiologically as is indicated by the rate and character of their growth on the same and different media and by the fact that they produce different degrees of infection on the same cereals and grasses.

6. The spores of the four biologic forms of *H. sativum* differ slightly in width and length and in the number of septa, as do also spores of a single form produced under different conditions.

7. Extreme variation of an individual biologic form of *H. sativum* under different conditions, and of different biologic forms under the same conditions makes it almost impossible to give a technical description of the species which would be generally applicable. A large number of spores must be measured to obtain reliable results, and any given dimension of spores is applicable only to a specific set of conditions.

8. *Helminthosporium sativum* has a very wide host range. Of the 134 species of cereals and grasses inoculated in the greenhouse with Form I, 98 became infected. Fifty-eight of these were susceptible and 40 were somewhat resistant. The remaining 36 appeared to be immune. Of the 66 species inoculated in the field, 57 became infected.

9. Wheat, barley, and rye are susceptible. The various species of oats and corn inoculated were practically all immune. The common grasses which are very susceptible are: *Setaria viridis*, *Hystrix patula*, *Chloris verticillata*, and various species of *Agropyron*, *Bromus*, *Elymus*, *Festuca*, *Hordeum*, and *Lolium*; those which are immune or extremely resistant are: *Phleum pratense*, *Arrhenatherum elatius*, *Alopecurus pratensis*, *Koeleria cristata*, and species of *Phalaris*, *Poa*, and *Agrostis*.

10. Field and greenhouse experiments with Form I proved that Marquis, Monad, and Red Durum wheats were extremely susceptible to root infection, while Kanred was resistant. Of the barleys, Lion, Bay Brewing, and Arequipa were very susceptible, while Manchuria,

Minn. No. 184, was quite resistant. Oats were either immune or highly resistant, while rye was slightly susceptible.

11. Stunting, excessive stooling, and spindling growth of susceptible plants were produced in the greenhouse under controlled conditions, as a result of artificial inoculation with *H. sativum*. This fungus also causes a shortening of the distance between the groundline and the base of the first leaf of infected plants.

12. Typical foot- and root-rot developed on wheat and barley when planted in infected soil in the greenhouse. The plants were spindling and dwarfed, and seedling blight was common. *H. sativum* was isolated from the diseased plants. Similar results were obtained by planting diseased seeds in clean soil.

13. *Helminthosporium sativum* overwinters as mycelium in the seed and on plant remains in the field. The mycelium has been shown to remain viable in seed for several years. Spores kept over winter outdoors in moist sand did not germinate in the spring, while spores under similar treatment in the laboratory were viable at the end of five months. Spores produced on a culture medium germinated freely at the end of twenty-three months. The mycelium also remained alive for many months when kept under varying conditions.

14. Initial infections in the field and greenhouse are due to seed infection and soil infestation. Secondary infections result from spores produced on primary lesions on cereals, from infected plants of the previous season, and from many diseased common grasses.

15. The fungus grows and sporulates not only on living plants, but also on dead and decaying plant parts.

16. The disease is extremely hard to control. Proper rotation and clean seed will reduce the prevalence of the disease but will not eliminate it. The use of resistant varieties seems to be the most promising means of control.

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## EXPLANATION OF PLATES

Plate I.

Badly infected Arequipa barley. Note numerous lesions. No heads developed on account of severe root infection. Photographed at harvest time. (Grown on Plant Pathology and Plant Breeding plot, 1921.)

Plate II.

Different degrees of infection on wheat, grown from black-tipped kernels in sterilized sand. *H. sativum* fruiting on seeds. Healthy plant on extreme right.

Plate III.

Fig. 1. Badly infected barley hybrid. Note excessive stooling. (Grown on Plant Pathology and Plant Breeding plot.)

Fig. 2. Effect of growing grasses in sand inoculated with *H. sativum*. From left to right.

A. *Agropyron tenerum:*
   Inoculated
   Check

B. *Hordeum murinum:*
   Inoculated
   Check

Plate IV.

Fig. 1. Black-tipped seeds of wheat sown on potato dextrose agar. Pure cultures of *H. sativum* developed from six and *Alternaria sp.* from the seventh.

Fig. 2. Photomicrograph of spores of *Helminthosporium sativum* developed on moist heads of wheat.

Fig. 3. Germinating spores.

Plate V.

Four biologic forms, I, II, III, and IV, of *H. sativum* on green bean agar. Compare with Plate VI.

Plate VI.

Four biologic forms, I, II, III, and IV, of *H. sativum* on one per cent potato dextrose agar. Compare with Plate V.

Plate VII.

Fig. 1. Second crop of wheat grown on soil previously infested with *H. sativum*. From left to right.

A. Monad:
   Sterilized soil
   Infested soil

B. Acme:
   Sterilized soil
   Infested soil

C. Kota:
   Sterilized soil
   Infested soil

Fig. 2. Effect of soil inoculated with *H. Sativum* on wheat. From left to right.

A. Kota:
Check
Inoculated

B. Red Durum:
Check
Inoculated

C. Marquis
Check
Inoculated

Plate VIII.

Second crop of wheat grown on soil previously infested with *H. sativum.* Same plants as in Plate VII, Fig. 1, except that Marquis is substituted for Monad. From left to right

A. Acme:
Infested soil
Sterilized soil

B. Marquis:
Infested soil
Sterilized soil

C. Kota:
Infested soil
Sterilized soil

Plate IX.

Different degrees of infection on two-rowed barley grown on peat soil in 1921. All the plants are the same age.

Plate X.

Result of growing wheat, barley, and oats on soil naturally infested with *H. sativum.* From left to right.

A. Marquis wheat:
Infested soil
Sterilized soil

B. Arequipa barley:
Infested soil
Sterilized soil

C. Manchuria barley:
Infested soil
Sterilized soil

D. Victory oats:
Infested soil
Sterilized soil

PLATE I

PLATE II

FIGURE 1


FIGURE 2
PLATE III

FIGURE 1

FIGURE 2

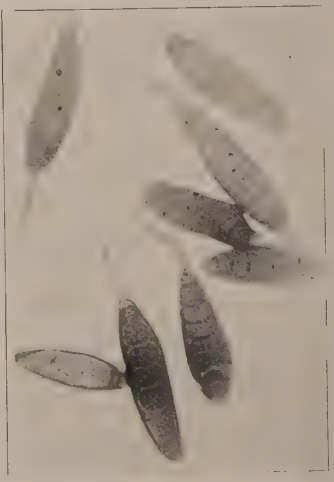

FIGURE 3

PLATE IV


PLATE V

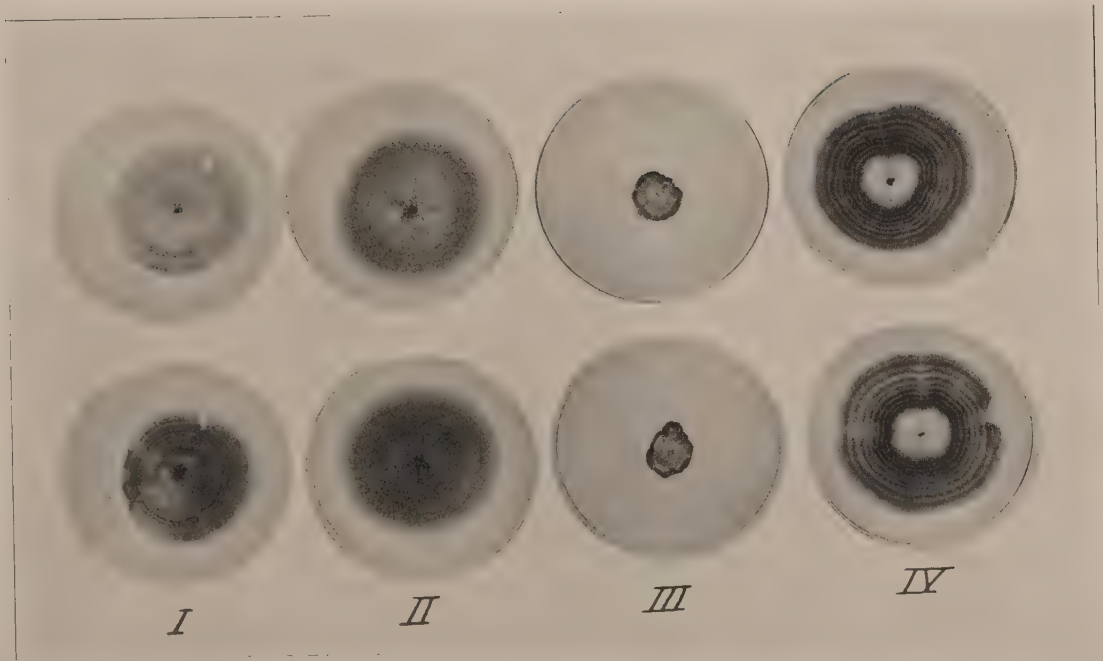


PLATE VI


FIGURE 1

FIGURE 2
PLATE VII


PLATE VIII

PLATE IX


PLATE X

STAFF OF DIVISION OF PLANT PATHOLOGY AND BOTANY

E. M. FREEMAN, Ph.D., Plant Pathologist and Botanist

*Section of Plant Pathology*

*E. C. STAKMAN, Ph.D., Plant Pathologist
J. G. LEACH, Ph.D., Assistant Plant Pathologist
*H. D. BARKER, M.S., Assistant Plant Pathologist
LOUISE DOSDALL, Ph.D., Mycologist
J. L. SEAL, M.S., Assistant Plant Pathologist
*J. J. CHRISTENSEN, M.S., Assistant in Plant Pathology
*D. L. BAILEY, M.S., Assistant in Plant Pathology
*E. B. LAMBERT, M.S., Assistant in Plant Pathology
*C. R. HURSH, B.S., Assistant in Plant Pathology
R. M NELSON, B.S., Assistant in Plant Pathology

*Section of Plant Physiology*

L. I. KNIGHT, Ph.D., Plant Physiologist
R. B. HARVEY, Ph.D., Associate Plant Physiologist
L. O. REGEIMBAL, B.S., Assistant in Plant Physiology
I. L. CONNERS, M.A., Assistant in Plant Physiology

*Section of Seed Laboratory*

A. H. LARSON, B.S., Seed Analyst
HENRY C. GILBERT, B.S., Assistant
RUBY URE, Assistant

* Coöperating with the Office of Cereal Investigations, Bureau of Plant Industry, United States Department of Agriculture.